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Distribution of 2,4,5-Trichlorophenoxyacetic Acid in the Mouse Fetus

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DISTRIBUTION OF 2,4,5-TRICHLOROPHENOXYACETIC ACID IN THE MOUSE FETUS

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SUMMARY

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) readily crosses the placenta in the mouse on gestational day 13. Concentrations of 2,4,5-T in maternal tissues and fetuses were obvious at 30 min, highest at 8 h, diminished by 24 h and almost entirely eliminated by 48 h. Autoradiographs of the whole fetus showed that initially 2,4,5-T was mainly in the highly vascularized tissues such as liver followed by the skin, eyes and ventricles of the brain. At 48 h, there was a general distribution of 2,4,5-T in the skeletal muscles. None was detected in the palate. The excretion rate in the mouse was approx. 60 μg/h; about 52% of the dose was recovered unchanged in the urine in 24 h.

Recent reports have shown that the herbicide 2,4,5-T was teratogenic in the mouse since it produced an increased incidence of cleft palates. Kidney and skeletal changes as well as growth retardation and delayed ossification have also been found [1,3,6,7]. Cleft palates can be produced with only a single administration of 2,4,5-T on gestational day 13 or 14, the time of palate closure. Previous work by Lindquist and Dencker [4] suggested that 2,4,5-T slowly crossed the placenta and dispersed evenly in the mouse fetus at mid-gestation. These studies employed autoradiographs of whole pregnant mice which made discrete observations of the fetus very difficult. We questioned whether 2,4,5-T accumulated in specific tissues of the fetus and whether it had a predelection for the palate. By use of autoradiographs, the distribution of 2,4,5-T was observed in the organs of the mouse fetus.

Abbreviations: DMSO, dimethylsulfoxide; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

METHOD

The non-radioactive 2,4,5-T was generously supplied by the Dow Chemical Co. of Midland, Michigan with a purity greater than 99% and a level of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or dioxin (TCDD) less than 0.05 ppm. The [1-^{14}C] 2,4,5-trichlorophenoxy acetic acid was purchased from the Amersham/Searle Co. with a specific activity of 30 mCi/mol (117 μCi/mg). The radiochemical purity was 98–99% by thin-layer chromatography.

Random bred CD-1 mice were purchased from the Charles River Labs., Inc. with known inseminati[illegible] dates. Detection of a vaginal plug indicated day zero of gestation. The mice were treated with 100 mg/kg of [^{14}C]-2,4,5-T on day 13 or daily from day 10–13 of gestation. Solutions of [^{14}C]-2,4,5-T in DMSO were administered by oral intubation using 0.1 ml/mouse/day. This solution contained 0.85 μCi/0.1 ml. Mice were sacrificed at the times specified. Serum and maternal tissue samples were collected for ^{14}C assay. Some fetuses of each litter were assayed for ^{14}C content and the rest used for autoradiographic studies. Tissues were solubilized in Soluene 100 (Packard Instrument Co., Inc.) and then combined with PPO and POPOP scintillation fluid. These samples were assayed for radioactivity using internal standards with a Beckman Scintillation counter; all results were corrected and presented as μg per 100 mg wet tissue weight. Chemical determination of 2,4,5-T was by the method of Courtney [2]. Fetuses for autoradiographic studies were embedded in paraffin, sectioned at 5 μm, coated with emulsion and developed for 2–4 months. The slides were stained with hematoxylin and eosin.

RESULT AND DISCUSSION

Tissues of pregnant CD-1 mice were assayed after single administration of [^{14}C] 2,4,5-T at the times shown in Table I. Maternal serum and tissue levels were highest at 8 h and declined steadily. At 48 h the compound was barely detectable. The fetuses and placentas showed peak concentrations at 8 h. The placentas always had higher concentrations than the fetuses.

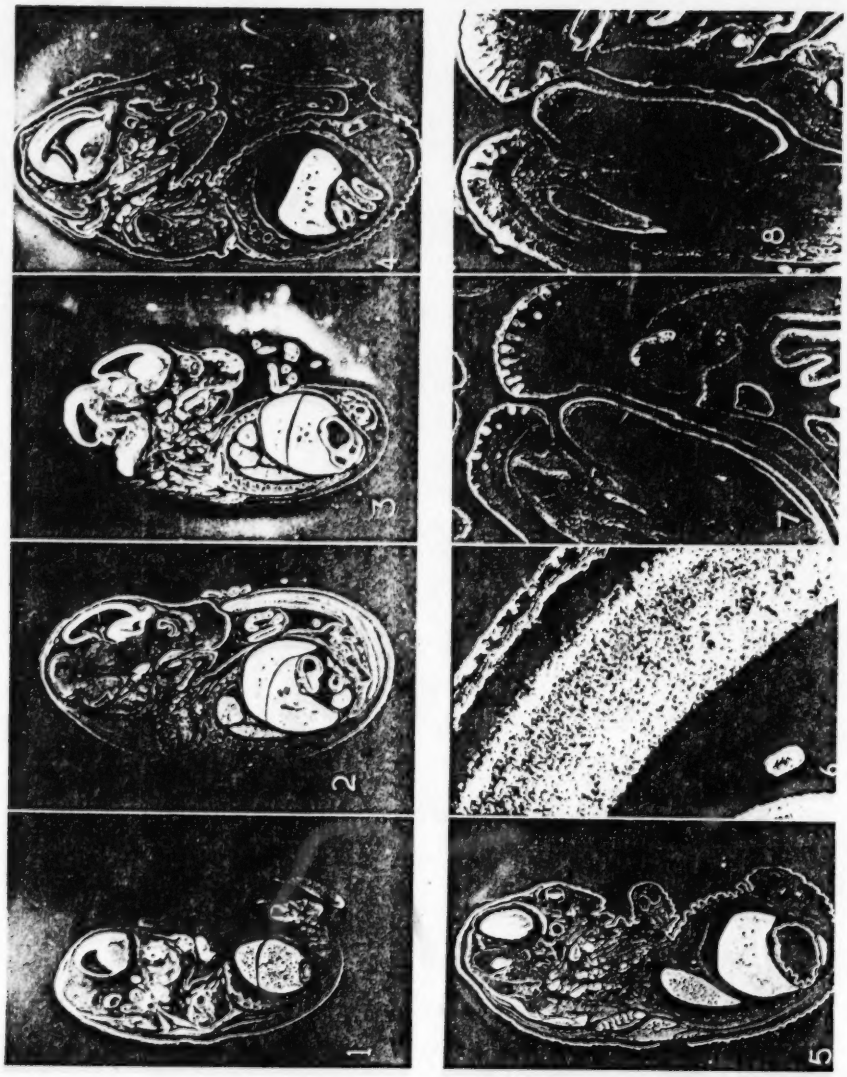
Another group of mice were treated with four daily doses from day 10–13 of gestation and sacrificed at 8 or 24 h after the last dose. These data are also presented in Table I. These multiple dosed mice were in the same stage of gestational development at sacrifice as the mice receiving a single dose. Four daily doses increased the maternal serum levels more than 10-fold while the liver, spleen, kidney, heart and uterus increased 2–3 times in concentration. In contrast, the skeletal muscle had approximately the same concentration after one dose as after four doses. All values for these tissues declined within 24 h but the decrease was not as marked as in the mice receiving a single dose. The fetuses of these multiple-dosed mice had approximately twice the concentration of [^{14}C] 2,4,5-T at 8 h compared to single-dosed mice. The placentas from these animals were assayed with membranes

TABLE I

CONCENTRATION OF [^{14}C]2,4,5-T IN MATERNAL TISSUES, PLACENTAS AND FETUSES OF CD-1 MICE ADMINISTERED 100 mg/kg ORALLY ON DAY 13 OR DAYS 10—13 OF GESTATION AND SACRIFICED AT TIMES INDICATED. RESULTS ARE MEANS ± SD IN μg/100 mg WET WEIGHT TISSUE

Time (h)	Number of mice	Serum $\bar{X}$ SD	Liver $\bar{X}$ SD	Spleen $\bar{X}$ SD	Kidney $\bar{X}$ SD	Heart $\bar{X}$ SD	Muscle $\bar{X}$ SD	Uterus $\bar{X}$ SD	Fetus $\bar{X}$ SD	Placenta $\bar{X}$ SD
[^{14}C]2,4,5-T Administered gestational day 13										
0.5	4	8.9±5.5	2.3±1.4	0.4±0.5	4.8±1.2	1.3±1.3	0.2±0.3	1.3±1.1	0.0	0.3±0.5
1.0	4	12.0±7.7	5.8±5.3	1.5±2.2	6.2±1.€	3.4±3.8	2.2±2.1	4.3±4.0	0.1±0.2	1.2±0.7
2.0	4	14.5±2.4	6±1.2	1.3±0.5	7.0±1.2	3.1±0.6	2.0±1.1	4.1±0.7	0.7±0.3	2.2±0.7
3.0	4	23.1±5.0	7.2±0.4	2.0±1.1	9.4±1.2	5.4±1.2	5.5±1.2	5.8±0.5	1.1±0.2	3.4±1.0
4.0	4	25.1±6.9	9.8±1.1	2.6±1.0	10.7±1.1	5.4±1.8	6.3±1.2	6.4±1.4	1.5±0.3	3.8±1.1
6.0	4	24.1±2.4	13.0±3.5	2.6±0.5	10.8±0.8	6.2±1.5	6.3±1.5	6.7±2.7	1.8±0.4	3.3±0.9
8.0	4	27.4±2.0	13.8±5.5	4.2±2.1	11.6±1.1	6.2±2.3	6.3±2.3	7.4±1.8	2.0±1.3	4.4±1.3
12.0	4	24.8±7.0	10.8±7.0	1.9±1.3	8.2±1.1	4.7±2.0	4.8±2.0	5.5±1.7	1.2±0.5	3.7±2.0
24.0	4	10.6±1.6	3.5±1.1	0.7±0.7	6.0±0.9	1.5±0.4	0.4±0.3	2.9±0.5	1.0±0.4	1.4±0.4
48.0	1	2.5 —	0.8 —	0.0 —	1.6 —	0.0 —	0.0 —	0.5	0.0	0.3±0.3
72.0	2	1.4 —	0.8 —	0.0 —	0.0 —	0.0 —	0.0 —	0.0 —	—	—
[^{14}C]2,4,5-T Administered gestational days 10—13										
8.0	4	383.7±372.5	31.6±17.3	8.0±3.4	18.2±6.3	17.7±4.9	8.2±2.5	15.1±2.1	4.8±1.1	11.7±2.0[a]
24.0	4	209.4±235.2	24.1±10.6	5.6±3.1	12.9±2.5	12.0±4.7	5.7±2.0	12.3±4.1	3.7±1.6	10.0±3.5[a]

[a] Assayed with membranes and yolk sacs.



Figs. 1—5. Autoradiographs of CD-1 mouse fetuses 30 min (Fig. 1), 2 h (Fig. 2), 8 h (Fig. 3), 24 h (Fig. 4), and 48 h (Fig. 5) after maternal intubation on day 13 of gestation of 100 mg/kg [^{14}C]2,4,5-T (light areas indicate radioactivity). Fig. 6. Details of skin (narrow structure) and brain at 8 h. Figs. 7 and 8. Details of mouth area at 8 and 48 h, respectively, showing no radioactivity in the palate. The cylindrical shaped structure in the center is the tongue.

and yolk sacs attached so a direct comparison with the single-dosed mice cannot be made. There was little decrease in the placental/yolk sac concentration at 24 h which may have helped maintain the fetal concentration.

The distribution and deposition of [^{14}C] 2,4,5-T in the fetuses from single-dosed mice was estimated by autoradiographs of sagittal sections of fetuses as shown in Figs. 1—5. Fig. 1 shows that the liver, eye, and ventricles have most of the compound at 30 min after treatment (white areas indicate [^{14}C] 2,4,5-T). Fig. 2 shows increased deposition in a fetus at 2 h. The [^{14}C] 2,4,5-T can be found in most tissues with the internal organs appearing to have the highest concentration. Fig. 3 shows a marked increase in the concentration of 2,4,5-T in the liver and brain of a fetus 8 h after treatment. By 24 h after treatment, [^{14}C] 2,4,5-T could be detected in all tissues at reduced levels (Fig. 4). At 48 h (Fig. 5), [^{14}C] 2,4,5-T was still present in the fetus. It should be noted that the skeletal muscles of the neck and thorax showed a fairly even distribution of the compound.

In Figs. 1—5, the skin has an obvious deposition of the compound. Waddell [8] suggested in his autoradiographic studies with other compounds that the skin deposition could be from the amniotic fluid. A higher magnification of the skin and brain at 8 h (Fig. 6) shows that [^{14}C] 2,4,5-T had penetrated throughout the skin. The skin could be a slow turnover depot for the compound since the [^{14}C] 2,4,5-T was still apparent at 48 h.

8 h after treatment, when the fetal concentration was greatest, there was no deposition of [^{14}C] 2,4,5-T in the palate as shown in Fig. 7. The tongue is the long cylindrical shaped object in the center, and a section of the amnion is next to the lips. The ethmoid cartilage had a greater deposition of [^{14}C] 2,4,5-T than the palate which is immediately adjacent to the cartilage. Fig. 8 shows a mouth region from a fetus at 48 h. There was no [^{14}C] 2,4,5-T activity in the palate although the lips showed a definite deposition. It is interesting to note that the compound is prominent in the follicles which produce the vibrissae (whiskers).

Daily urines were collected from the multiple-treated mice. Spectrophotometric scans of the urine extract showed only 2,4,5-T to be present. At 8, 24, 48, 72 and 96 h after the last dose, the cumulative mg of the total dose excreted per mouse were 0.43, 1.49, 2.60, 2.40 and 3.04, respectively. Mice excreted 52% of the total dose at 24 and 48 h. After 48 h the excretion rate appeared to plateau. In general, the mice excreted 60 μg/h/mouse (1500 μg/h/kg of mouse).

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